

CLAIM AMENDMENTS

Claims 1-59 (canceled)

Claim 60 (currently amended)      A method for the amplification of at least one target nucleic acid sequence of interest, comprising, for each target nucleic acid sequence of interest:

(a)      contacting upstream and downstream oligonucleotide ligation probes with the target nucleic acid sequence such that the 3' terminus of the upstream probe is juxtaposed to the 5' terminus of the downstream probe while both the upstream and downstream probes are in contact with the target nucleic acid sequence;

(b)      ligating together the upstream and downstream probes at their respective juxtaposed termini to form a ligated target probe template; and

(c)      using the ligated target probe template in a strand displacement amplification ("SDA") reaction to form amplicons of the ligated target probe template, wherein the strand displacement reaction utilizes at least a first SDA primer, each SDA primer comprising a restriction endonuclease sequence upstream of a sequence specific for the ligated target probe template on the 5' region of the primer, and no bumper primers are used in the strand displacement amplification, wherein steps (a), (b), and (c) ~~occur~~ exist in solution at the same time.

Claims 61-77 (canceled)

Claim 78 (currently amended)      A method for the amplification of at least one target nucleic acid sequence of interest, comprising, for each target nucleic acid sequence of interest:

- (a) contacting upstream and downstream oligonucleotide ligation probes with the target nucleic acid sequence such that the 3' terminus of the upstream probe is juxtaposed to the 5' terminus of the downstream probe while both the upstream and downstream probes are in contact with the target nucleic acid sequence;
- (b) ligating together the upstream and downstream probes at their respective juxtaposed termini to form a ligated target probe template; and
- (c) using the ligated target probe template in a strand displacement amplification ("SDA") reaction to form amplicons of the ligated target probe template, wherein the strand displacement reaction utilizes at least a first and a second SDA primer, each SDA primer comprising a restriction endonuclease sequence upstream of a sequence specific for the ligated target probe template on the 5' region of the primer, and the strand displacement reaction utilizes an unequal effective concentration ratio of the first SDA primer to the second SDA primer, wherein the first and second SDA primers are different primers and form a set of primers, and wherein unequal populations of complementary first and second amplified strands of the target nucleic acids are formed,
- wherein steps (a), (b), and (c) occur at the same time.

Claim 79 (amended) The method of claim 78 wherein the unequal effective concentration ratio is obtained by providing at least one SDA primer of the set in molar excess as compared to the other SDA primer in the set.

Claim 80 (previously added) The method of claim 79 wherein the SDA primer that is in molar excess is labeled.

Claim 81 (previously added) The method of claim 80 wherein the label is a fluorescent label.

Claim 82 (previously added) The method of claim 81 wherein the fluorescent label is selected from the group consisting of Bodipy-derivatives, Cyanine-derivatives, fluorescein-derivatives, and rhodamine-derivatives.

Claim 83 (previously added) The method of claim 80 wherein the label is a chemiluminescence label.

Claim 84 (previously added) The method of claim 80 wherein the label is an electrochemiluminescence label.

Claim 85 (previously added) The method of claim 80 wherein the label is biotin.

Claim 86 (currently amended) The method of claim 78 wherein the unequal effective concentration ratio is obtained by providing a competitor in the amplification reaction ~~for to~~ either the first SDA primer or the second SDA primer, and wherein the competitor binds to the ligated target probe template and is selected from the group consisting of: non-extendable competitors, non-cleavable competitors, and competitors which are non-extendable and non-cleavable.

Claim 87 (previously added) The method of claim 86 wherein the competitor is anchored to a substrate.

Claim 88 (previously added) The method of claim 86 wherein the competitor is in solution.

Claim 89 (previously added) The method of claim 86 wherein the competitor is a non-extendable competitor.

Claim 90 (previously added) The method of claim 89 wherein the competitor comprises a non-extendable 3' modification selected from the group consisting of: a 3' terminal base mis-match, a 3' dideoxy nucleic acid, and a blocking group attached to the 3' hydroxy group of the 3' terminal nucleic acid.

Claim 91 (previously added) The method of claim 86 wherein the competitor is a non-cleavable competitor.

Claim 92 (previously added) The method of claim 91 wherein the non-cleavable competitor comprises a nucleic acid selected from the group consisting of: methylated nucleic acids and phosphorothiolated nucleic acid.

Claim 93 (previously added) The method of claim 91 wherein the non-cleavable competitor comprises a modified nucleic acid selected from the group consisting of 2'-deoxyadenosine 5'-O-(1-thiotriphosphate), 5-methyldeoxycytidine 5'-triphosphate, 2'-deoxyuridine 5'-triphosphate, and 7-deaza-2'-deoxyguanosine 5'-triphosphate.

Claim 94 (previously added) The method of claim 86 wherein the SDA primer which the competitor does not compete with is labeled.

Claim 95 (previously added) The method of claim 94 wherein the label is a fluorescent label.

Claim 96 (previously added) The method of claim 95 wherein the fluorescent label is selected from the group consisting of Bodipy-derivatives, Cyanine-derivatives, fluorescein-derivatives, and rhodamine-derivatives.

Claim 97 (previously added) The method of claim 94 wherein the label is a chemiluminescence label.

Claim 98 (previously added) The method of claim 94 wherein the label is an electrochemiluminescence label.

Claim 99 (previously added) The method of claim 94 wherein the label is biotin.

Claim 100 (currently amended) A method for the amplification of at least one target nucleic acid sequence of interest, comprising, for each target nucleic acid sequence of interest:

(a) contacting upstream and downstream oligonucleotide ligation probes with the target nucleic acid sequence such that the 3' terminus of the upstream probe is juxtaposed to the 5' terminus of the downstream probe while both the upstream and downstream probes are in contact with the target nucleic acid sequence;

(b) ligating together the upstream and downstream probes at their respective juxtaposed termini to form a ligated target probe template; and

(c) using the ligated target probe template in a strand displacement amplification (“SDA”) reaction to form amplicons of the ligated target probe template, wherein the strand displacement reaction utilizes at least a first SDA primer and a second SDA primer, each SDA primer comprising a restriction endonuclease sequence upstream of a sequence specific for the ligated target probe template on the 5' region of the primer, wherein the first and second SDA primers are different primers,

wherein steps (a), (b), and (c) occur at the same time and at least one of the first and second SDA primers is labeled.

Claim 101 (previously added) The method of claim 100 wherein the label is a fluorescent label.

Claim 102 (previously added) The method of claim 101 wherein the fluorescent label is selected from the group consisting of Bodipy-derivatives, Cyanine-derivatives, fluorescein-derivatives, and rhodamine-derivatives.

Claim 103 (previously added) The method of claim 100 wherein the label is a chemiluminescence label.

Claim 104 (previously added) The method of claim 100 wherein the label is an electrochemiluminescence label.

Claim 105 (previously added) The method of claim 100 wherein the label is biotin.

Claim 106 (currently amended) A method for the amplification of at least one target nucleic acid sequence of interest, comprising, for each target nucleic acid sequence of interest:

(a) contacting upstream and downstream oligonucleotide ligation probes with the target nucleic acid sequence such that the 3' terminus of the upstream probe is juxtaposed to the 5' terminus of the downstream probe while both the upstream and downstream probes are in contact with the target nucleic acid sequence;

(b) ligating together the upstream and downstream probes at their respective juxtaposed termini to form a ligated target probe template; and

(c) using the ligated target probe template in a strand displacement amplification ("SDA") reaction to form amplicons of the ligated target probe template, wherein the strand displacement reaction utilizes at least a first SDA primer, each SDA primer comprising a restriction endonuclease sequence upstream of a sequence specific for the ligated target probe template on the 5' region of the primer,

wherein steps (a), (b), and (c) ~~occur~~ exist in solution at the same time,

wherein allele specific strand displacement amplification is carried out for target nucleic acids of interest which encode a gene with two or more known alleles,

wherein the upstream and downstream ligation probe sequences are selected to be specific for a particular allele of the gene, so that the ligation probes contact, proximate the 3' end of the upstream probe or proximate the 5' end of the downstream probe, a ~~portion~~ nucleotide sequence of the target nucleic acid sequence which is determinative of the particular allele,

wherein, if the target nucleic acid does not contain the sequence determinative of the allele for which the ligation probes are selected, the juxtaposed terminus of one of the probes is misaligned, so that, in step (b), the ligation probes are ligated only if the target nucleic acid sequence contains the allele determinative sequence, and

wherein, in step (c), amplicons are produced by strand displacement amplification if the allele determinative sequence is contained within the target nucleic acid sequence.

Claim 107 (previously added)The method of claim 106 wherein the upstream probe is particular for the allele determinative sequence, and is not complementary for other alleles at the ultimate 3' nucleic acid of the upstream probe.

Claim 108 (previously added)The method of claim 106 wherein the upstream probe is particular for the allele determinative sequence, and is not complementary for other alleles at the penultimate 3' nucleic acid of the upstream probe.

Claim 109 (previously added)The method of claim 106 wherein the downstream probe is particular for the allele determinative sequence, and is not complementary for other alleles at the ultimate 5' nucleic acid of the downstream probe.

Claim 110 (previously added)The method of claim 106 wherein the downstream probe is particular for the allele determinative sequence, and is not complementary for other alleles at the penultimate 5' nucleic acid of the downstream probe.



Claim 111 (currently amended) A method for the amplification of at least one target

nucleic acid sequence of interest, comprising, for each target nucleic acid sequence of interest:

(a) contacting upstream and downstream oligonucleotide ligation probes with the target nucleic acid sequence such that the 3' terminus of the upstream probe is juxtaposed to the 5' terminus of the downstream probe while both the upstream and downstream probes are in contact with the target nucleic acid sequence, wherein the upstream and downstream oligonucleotide ligation probes are initially incapable of being ligated together;

(b) ligating together the upstream and downstream probes at their respective juxtaposed termini to form a ligated target probe template; and

(c) using the ligated target probe template in a strand displacement amplification ("SDA") reaction to form amplicons of the ligated target probe template, wherein the strand displacement reaction utilizes at least a first SDA primer, wherein each SDA primer comprising a restriction endonuclease sequence upstream of a sequence specific for the ligated target probe template on the 5' region of the primer,

wherein steps (a), (b), and (c) ~~occur~~ exist in solution at the same time and ~~the upstream and downstream oligonucleotide ligation probes are initially incapable of being ligated together,~~ the method further comprising rendering capable of being ligated together the upstream and downstream oligonucleotide ligation probes prior to ligating the probes together in step (b).

Claim 112 (previously added) The method of claim 111 wherein the rendering comprises removing one or more terminal nucleotides from the oligonucleotide probes.

Claim 113 (previously added)The method of claim 112 wherein one or more terminal nucleotides are removed from the 3' terminus of the upstream oligonucleotide ligation probe.

Claim 114 (previously added)The method of claim 113 wherein one or more terminal nucleotides are removed by an endonuclease.

Claim 115 (previously added)The method of claim 114 wherein the endonuclease is Endonuclease IV.

Claim 116 (previously added)The method of claim 112 wherein one or more terminal nucleotides are removed from the 5' terminus of the downstream oligonucleotide ligation probe.

Claim 117 (previously added)The method of claim 116 wherein one or more terminal nucleotides are removed by an exonuclease.

Claim 118 (previously added)The method of claim 117 wherein the exonuclease is a DNA polymerase.

Claim 119 (previously added)The method of claim 112 wherein one or more terminal nucleotides that are removed are non-complementary to the target sequence.

Claims 120-132 (canceled)

Claim 133 (currently amended)      A method for the amplification of at least one target

nucleic acid sequence of interest from at least one sample, using an electronically addressable microchip, comprising, for each target nucleic acid sequence of interest:

- (a)      contacting upstream and downstream oligonucleotide ligation probes with the target nucleic acid sequence such that the 3' terminus of the upstream probe is juxtaposed to the 5' terminus of the downstream probe while both the upstream and downstream probes are in contact with the target nucleic acid sequence;
- (b)      ligating together the upstream and downstream probes at their respective juxtaposed termini to form a ligated target probe template; and
- (c)      using the ligated target probe template in a strand displacement amplification (“SDA”) reaction to form amplicons of the ligated target probe template, wherein the strand displacement reaction utilizes at least a first SDA primer, each SDA primer comprising a restriction endonuclease sequence upstream of a sequence specific for the ligated target probe template on the 5' region of the primer, and no bumper primers are used in the strand displacement amplification, wherein at least one nucleic acid selected from group consisting of target sequences, ligated target probe templates, and amplicons is electronically addressed in a step (d) to any of a plurality of capture sites on the bioelectronic microchip.

Claims 134-135 (canceled)

Claim 136 (currently amended)      A method for the amplification of at least one target nucleic acid sequence of interest from at least one sample, using an electronically addressable microchip, comprising, for each target nucleic acid sequence of interest:

- (a) contacting upstream and downstream oligonucleotide ligation probes with the target nucleic acid sequence such that the 3' terminus of the upstream probe is juxtaposed to the 5' terminus of the downstream probe while both the upstream and downstream probes are in contact with the target nucleic acid sequence;
- (b) ligating together the upstream and downstream probes at their respective juxtaposed termini to form a ligated target probe template; and
- (c) using the ligated target probe template in a strand displacement amplification ("SDA") reaction to form amplicons of the ligated target probe template, wherein the strand displacement reaction utilizes at least a first SDA primer and a second SDA primer, the first and second SDA primers being different primers, each SDA primer comprising a restriction endonuclease sequence upstream of a sequence specific for the ligated target probe template on the 5' region of the primer, and the first and second SDA primers are contained within a branched primer structure, having the capacity to support strand displacement amplification, and the branched primer is attached to ~~the~~ a capture site,
- wherein at least one nucleic acid selected from group consisting of target sequences, ligated target probe templates, and amplicons is electronically addressed in a step (d) to any of a plurality of capture sites on the bioelectronic microchip.

Claim 137 (previously added)The method of claim 136 wherein the branched primer is attached to the capture site through a biotin/streptavidin interaction.

Claim 138 (previously added)The method of claim 136 wherein the branched primer is attached to the capture site through a covalent bond.

Claim 139 (previously added) The method of claim 136 wherein the branched primer is formed by attaching the first and second SDA primers to an amino acid.

Claim 140 (previously added) The method of claim 139 wherein the amino acid is lysine.

Claim 141 (previously added) The method of claim 136 wherein the branched primer formed by attaching the first and second SDA primers to a spacer molecule selected from the group consisting of polyethylene glycol polymers, polyamino acids, and a polyfunctional amino acid which has been derivatized with two nucleic acid oligomers.

Claims 142-159 (canceled)

Claim 160 (currently amended) A method for the amplification of at least one target nucleic acid sequence of interest from at least one sample, using an electronically addressable microchip, comprising, for each target nucleic acid sequence of interest:

(a) contacting upstream and downstream oligonucleotide ligation probes with the target nucleic acid sequence such that the 3' terminus of the upstream probe is juxtaposed to the 5' terminus of the downstream probe while both the upstream and downstream probes are in contact with the target nucleic acid sequence;

(b) ligating together the upstream and downstream probes at their respective juxtaposed termini to form a ligated target probe template; and

(c) using the ligated target probe template in a strand displacement amplification

("SDA") reaction to form amplicons of the ligated target probe template, wherein the strand displacement reaction utilizes at least a first SDA primer, each SDA primer comprising a restriction endonuclease sequence upstream of a sequence specific for the ligated target probe template on the 5' region of the primer,

wherein at least one nucleic acid selected from group consisting of target sequences, ligated target probe templates, and amplicons is electronically addressed in a step (d) to any of a plurality of capture sites on the bioelectronic microchip and the upstream and downstream oligonucleotide ligation probes are initially incapable of being ligated together, the method further comprising rendering capable of being ligated together the upstream and downstream oligonucleotide ligation probes prior to ligating the probes together in step (b).

Claim 161 (previously added)The method of claim 160 wherein the rendering comprises removing one or more terminal nucleotides from the oligonucleotide probes.

Claim 162 (previously added)The method of claim 161 wherein one or more terminal nucleotides are removed from the 3' terminus of the upstream oligonucleotide ligation probe.

Claim 163 (previously added)The method of claim 162 wherein one or more terminal nucleotides are removed by an endonuclease.

Claim 164 (previously added)The method of claim 163 wherein the endonuclease is Endonuclease IV.

Claim 165 (previously added) The method of claim 161 wherein one or more terminal nucleotides are removed from the 5' terminus of the downstream oligonucleotide ligation probe.

Claim 166 (previously added) The method of claim 165 wherein one or more terminal nucleotides are removed by an exonuclease.

Claim 167 (previously added) The method of claim 166 wherein the exonuclease is a DNA polymerase.

Claim 168 (previously added) The method of claim 161 wherein one or more terminal nucleotides that are removed are non-complementary to the target sequence.

Claims 169-175 (canceled)

Claim 176 (currently amended) A method for the amplification of at least one target nucleic acid sequence of interest from at least one sample, using an electronically addressable microchip, comprising, for each target nucleic acid sequence of interest:

(a) contacting upstream and downstream oligonucleotide ligation probes with the target nucleic acid sequence such that the 3' terminus of the upstream probe is juxtaposed to the 5' terminus of the downstream probe while both the upstream and downstream probes are in contact with the target nucleic acid sequence;

(b) ligating together the upstream and downstream probes at their respective juxtaposed termini to form a ligated target probe template; and

(c) using the ligated target probe template in a strand displacement amplification ("SDA") reaction to form amplicons of the ligated target probe template, wherein the strand displacement reaction utilizes at least a first SDA primer and a second SDA primer, each SDA primer comprising a restriction endonuclease sequence upstream of a sequence specific for the ligated target probe template on the 5' region of the primer, and the strand displacement amplification utilizes an unequal effective concentration ratio of the first SDA primer to the second SDA primer, wherein the first and second SDA primers are different primers and form a set of primers, and wherein unequal populations of complementary first and second amplified strands of the target nucleic acids are formed,

wherein at least one nucleic acid selected from group consisting of target sequences, ligated target probe templates, and amplicons is electronically addressed in a step (d) to any of a plurality of capture sites on the bioelectronic microchip.

Claim 177 (currently amended) The method of claim 176 wherein the unequal effective concentration ratio is obtained by providing at least one SDA primer of the set of primers in molar excess as compared to the other SDA primer in the set of primers.

Claim 178 (previously added) The method of claim 177 wherein the SDA primer that is in molar excess is labeled.



Claim 179 (previously added) The method of claim 178 wherein the label is a fluorescent label.

Claim 180 (previously added) The method of claim 179 wherein the fluorescent label is selected from the group consisting of Bodipy-derivatives, Cyanine-derivatives, fluorescein-derivatives, and rhodamine-derivatives.

Claim 181 (previously added) The method of claim 178 wherein the label is a chemiluminescence label.

Claim 182 (previously added) The method of claim 178 wherein the label is an electrochemiluminescence label.

Claim 183 (previously added) The method of claim 178 wherein the label is biotin.

Claim 184 (currently amended) The method of claim 176 wherein the unequal effective concentration ratio is obtained by providing a competitor in the amplification reaction ~~for to~~ either the first SDA primer or the second SDA primer, and wherein the competitor binds to the ligated target probe template and is selected from the group consisting of: non-extendable competitors, non-cleavable competitors, and competitors which are non-extendable and non-cleavable.

Claim 185 (previously added) The method of claim 184 wherein the competitor is anchored to a substrate.

Claim 186 (previously added)The method of claim 184 wherein the competitor is in solution.

Claim 187 (previously added)The method of claim 184 wherein the competitor is a non-extendable competitor.

Claim 188 (previously added)The method of claim 187 wherein the competitor comprises a non-extendable 3' modification selected from the group consisting of: a 3' terminal base mis-match, a 3' dideoxy nucleic acid, and a blocking group attached to the 3' hydroxy group of the 3' terminal nucleic acid.

Claim 189 (previously added)The method of claim 184 wherein the competitor is a non-cleavable competitor.

Claim 190 (previously added)The method of claim 189 wherein the non-cleavable competitor comprises a nucleic acid selected from the group consisting of: methylated nucleic acids and phosphorothiolated nucleic acid.

Claim 191 (previously added)The method of claim 189 wherein the non-cleavable competitor comprises a modified nucleic acid selected from the group consisting of 2'deoxyadenosine 5'-O-(1-thiotriphosphate), 5-methyldeoxycytidine 5'-triphosphate, 2'-deoxyuridine 5'-triphosphate, and 7-deaza-2'-deoxyguanosine 5'-triphosphate.

Claim 192 (previously added) The method of claim 184 wherein the SDA primer which the competitor does not compete with is labeled.

Claim 193 (previously added) The method of claim 192 wherein the label is a fluorescent label.

Claim 194 (previously added) The method of claim 193 wherein the fluorescent label is selected from the group consisting of Bodipy-derivatives, Cyanine-derivatives, fluorescein-derivatives, and rhodamine-derivatives.

Claim 195 (previously added) The method of claim 192 wherein the label is a chemiluminescence label.

Claim 196 (previously added) The method of claim 192 wherein the label is an electrochemiluminescence label.

Claim 197 (previously added) The method of claim 192 wherein the label is biotin.

Claims 198-203 (canceled)

Claim 204 (currently amended) A method for the amplification of at least one target nucleic acid sequence of interest from at least one sample, using an electronically addressable microchip, comprising, for each target nucleic acid sequence of interest:

- (a) contacting upstream and downstream oligonucleotide ligation probes with the target nucleic acid sequence such that the 3' terminus of the upstream probe is juxtaposed to the 5' terminus of the downstream probe while both the upstream and downstream probes are in contact with the target nucleic acid sequence;
- (b) ligating together the upstream and downstream probes at their respective juxtaposed termini to form a ligated target probe template; and
- (c) using the ligated target probe template in a strand displacement amplification ("SDA") reaction to form amplicons of the ligated target probe template, wherein the strand displacement reaction utilizes at least a first SDA primer, each SDA primer comprising a restriction endonuclease sequence upstream of a sequence specific for the ligated target probe template on the 5' region of the primer,
- wherein at least one nucleic acid selected from group consisting of target sequences, ligated target probe templates, and amplicons is electronically addressed in a step (d) to any of a plurality of capture sites on the bioelectronic microchip,
- wherein allele specific strand displacement amplification is carried out for target nucleic acids of interest which encode a gene with two or more known alleles,
- wherein the upstream and downstream ligation probe sequences are selected to be specific for a particular allele of the gene, so that the ligation probes contact, proximate the 3' end of the upstream probe or proximate the 5' end of the downstream probe, a ~~portion~~ nucleotide sequence of the target nucleic acid sequence which is determinative of the particular allele,
- wherein, if the target nucleic acid does not contain the sequence determinative of the allele for which the ligation probes are selected, the juxtaposed terminus of one of the probes is

misaligned, so that, in step (b), the ligation probes are ligated only if the target nucleic acid sequence contains the allele determinative sequence, and

wherein, in step (c), amplicons are produced by strand displacement amplification if the allele determinative sequence is contained within the target nucleic acid sequence.

Claim 205 (previously added)The method of claim 204 wherein the gene has two known alleles, and the target nucleic acids are addressed to two capture sites.

Claim 206 (previously added)The method of claim 204 wherein the gene has multiple known alleles, and the target nucleic acids are addressed to multiple capture sites.

Claim 207 (previously added)The method of claim 204 wherein the upstream probe is particular for the allele determinative sequence, and is not complementary for other alleles at the ultimate 3' nucleic acid of the upstream probe.

Claim 208 (previously added)The method of claim 204 wherein the upstream probe is particular for the allele determinative sequence, and is not complementary for other alleles at the penultimate 3' nucleic acid of the upstream probe.

Claim 209 (previously added)The method of claim 204 wherein the downstream probe is particular for the allele determinative sequence, and is not complementary for other alleles at the ultimate 5' nucleic acid of the downstream probe.

Claim 210 (previously added) The method of claim 204 wherein the downstream probe is particular for the allele determinative sequence, and is not complementary for other alleles at the penultimate 5' nucleic acid of the downstream probe.